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Detection of Methicillin-Resistant *Staphylococcus aureus* Strains Resistant to Multiple Antibiotics and Carrying the Panton-Valentine Leukocidin Genes in an Algiers Hospital

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Forty-five Panton-Valentine leukocidin (PVL)-positive, methicillin-resistant *Staphylococcus aureus* strains were isolated in Algeria between 2003 and 2004; 18 isolates were isolated in the community and 27 in a hospital. Five PVL-positive hospital isolates were resistant to multiple antibiotics, including ofloxacin and gentamicin for three isolates.

First identified in the 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) was initially considered a nosocomial pathogen. However, in recent years, increasing numbers of MRSA strains have been isolated worldwide from patients with community-acquired infections (1, 5, 7–10, 12, 24, 28). Two main types of MRSA now circulate in the community: (i) hospital-derived strains (H-MRSA) that infect patients with risk factors such as recent hospitalization, surgery, underlying chronic diseases, or immunosuppression and (ii) strains arising de novo in the community (C-MRSA) and infecting patients with no established risk factors. C-MRSA strains tend to be susceptible to more antibiotics than H-MRSA strains, but C-MRSA strains from the United States were reported to be resistant to erythromycin and fluoroquinolones in 2005 (26). C-MRSA strains also harbor specific virulence genes associated with skin and soft-tissue infections, including the Panton-Valentine leukocidin (PVL) genes. Other virulence factors, such as superantigenic toxins, had been detected also in some C-MRSA strains (26, 28). C-MRSA strains have a small methicillin resistance cassette (SCC*mec* type IV or V), whereas most H-MRSA strains have a larger cassette (SCC*mec* type I or II) (6, 20, 24, 28).

Epidemiological data on MRSA in Africa are scarce. The prevalence of MRSA was determined in eight African countries between 1996 and 1997 and was relatively high in Nigeria, Kenya, and Cameroon (21 to 30%) and below 10% in Tunisia and Algeria (15). In Algeria, the rate increased to 14% in 2001 (25). PVL-positive C-MRSA strains are thought to circulate in Algeria, based on reports of infections in patients originating from Algeria but generally diagnosed in Europe (10, 17).

Mustapha Pacha hospital is the largest hospital in Algeria, with 1,800 beds. Between 2003 and 2004, 614 *S. aureus* strains were cultured and identified by the laboratory and 204 (33.2%) were found to be resistant to methicillin. Here we determined the toxin expression and genomic characteristics of 61 randomly selected MRSA strains isolated in this hospital from patients with community-acquired infections (20 cases) or hospital-acquired infections (41 cases).

Species identification was based on colony morphology and microscopic examination and on the results of the coagulase rabbit plasma and Staphyslide agglutination tests (bioMérieux). Antimicrobial susceptibility was determined by the disk diffusion

TABLE 1. Antibiotic resistance profiles of PVL-positive MRSA detected at Mustapha Pacha Hospital

Antibiotic resistance profile[a]	*agr* type	Community-acquired isolates (n = 18)	Hospital-acquired isolates (n = 27)	Total (n = 45)
OXA, KAN, TET, ERY, CLI	2	0	1	1
OXA, KAN, TET, FU	3	12	11	23
OXA, KAN, TET, ERY, FU	3	1	4	5
OXA, KAN	3	1	3	4
OXA, KAN, ERY	3	0	2	2
OXA, KAN, ERY, FU	3	1	0	1
OXA, KAN, TET, CLI, FU	3	1	0	1
OXA, KAN, TET, FU, OFX	3	1	0	1
OXA, KAN, FU, OFX	3	1	0	1
OXA, TET, FU	3	0	1	1
OXA, KAN, ERY, CLI, PT, FU, OFX[b]	3	0	1	1
OXA, KAN, TET, ERY, CLI, CHL, FU, OFX[b]	3	0	1	1
OXA, KAN, GEN, FU, OFX[b]	3	0	1	1
OXA, KAN, GEN, ERY, CLI, PT, FU, OFX[b]	3	0	1	1
OXA, KAN, GEN, FU, OFX, RIF[b]	3	0	1	1

[a] OXA, oxacillin; KAN, kanamycin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; FU, fusidic acid; OFX, ofloxacin; PT, pristinamycin; CHL, chloramphenicol; GEN, gentamicin; RIF, rifampin.

[b] Multidrug-resistant MRSA strains were isolated in the dermatology department from patients with skin infections.

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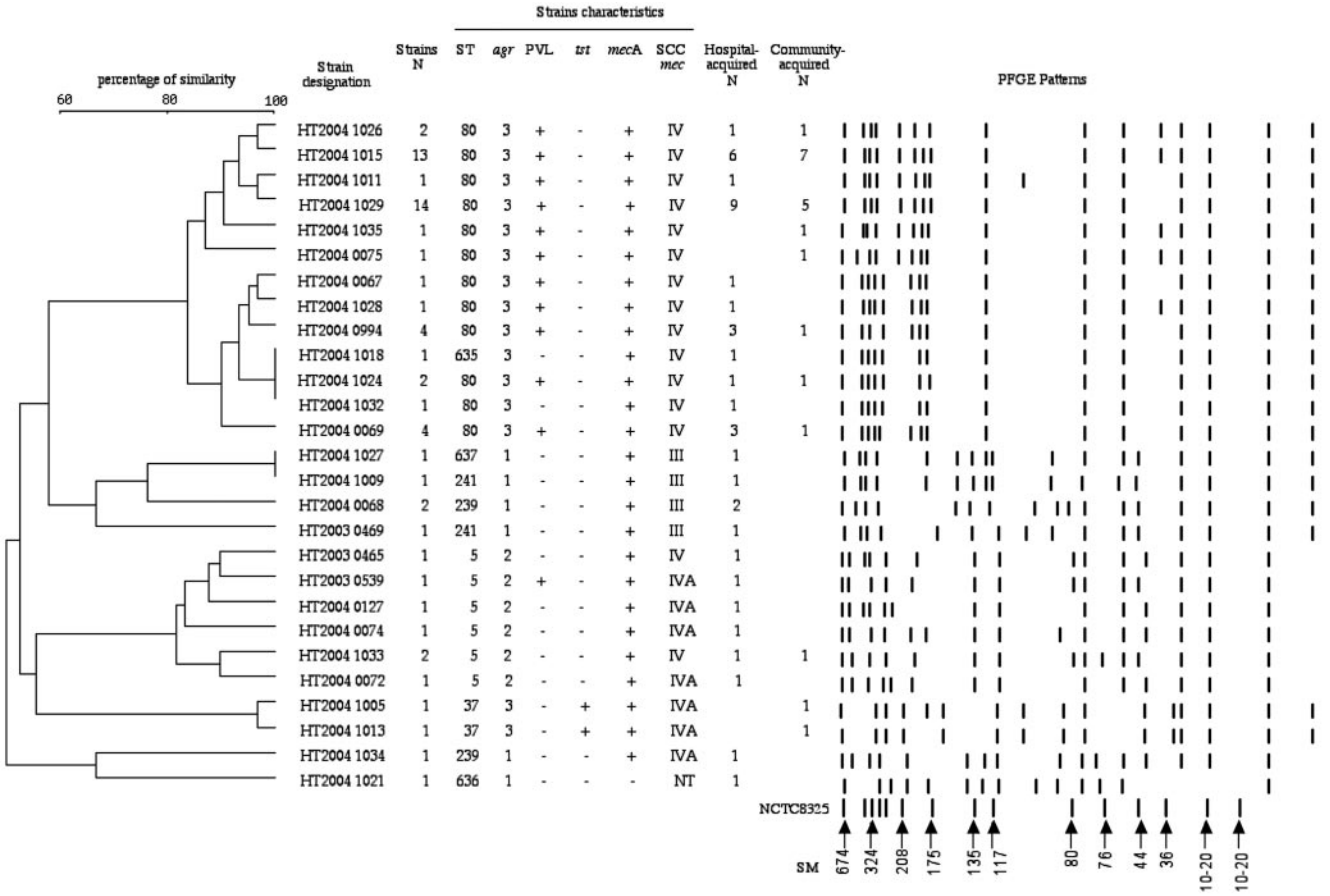

FIG. 1. Dendrogram of Algerian H-MRSA and C-MRSA isolates with genetic characteristics such as ST, *agr* allele group, toxin gene profile (PVL and *tst* genes), *mecA* gene, SCC*mec* typing, and PFGE patterns. Fragment sizes (in kilobases) of reference strain NCTC8325 are indicated at the bottom. SM, size markers.

method according to Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical and Laboratory Standards) recommendations (21a). The *mecA* gene coding for methicillin resistance was detected by PCR as described elsewhere (19). The toxic shock syndrome toxin gene (*tst*), the PVL genes (*lukS*-PV and *lukF*-PV), and accessory gene regulator alleles (*agr* types 1 to 4) were detected by PCR as previously described (10, 13, 14, 18). Pulsed-field gel electrophoresis of genomic DNA was performed after SmaI digestion as previously described (10), and multilocus sequence typing was performed as described by Enright et al. (11).

The most frequently detected toxin genes were the PVL genes (45/61 isolates, 72%). The PVL genes were harbored by a major clone accounting for 44 of the 45 PVL-positive isolates. This clone had the following characteristics: sequence type 80 (ST80), *agr* type 3, and SCC*mec* type IV. The PFGE patterns of these 44 isolates showed more than 80% similarity (Fig. 1). Forty-three isolates (97.6%) were resistant to kanamycin, 38 (86%) were resistant to fusidic acid, 32 (73%) were resistant to tetracycline, 11 (25%) were resistant to erythromycin, 7 (16%) were resistant to ofloxacin, 5 (11.3%) were resistant to clindamycin, 3 (7%) were resistant to gentamicin, 2 (4.5%) were resistant to pristinamycin, 1 (2.3%) was resistant to chloramphenicol, and 1 (2.3%) was resistant to rifampin (Table 1). Another PVL-positive clone was represented by a single isolate (ST5, *agr* type 2, SCC*mec* type IVA). PVL-positive isolates caused 18 (86%) of the 21 community-acquired infections and 27 (67.5%) of the 40 hospital-acquired infections.

PVL-positive MRSA strains thus appear to be widespread in Mustapha Pacha Hospital, although it is possible that the patients concerned were nasal carriers on admission (23). The high incidence of H-MRSA infection associated with PVL-positive *S. aureus* strains suggests the occurrence of a hospital outbreak due to this clone. Health care workers can also disseminate MRSA (2, 22, 27). The carriage rate of PVL-positive MRSA in the Algiers community is not known, but a high carriage rate might lead to an increase in hospital infections.

PVL-positive ST80 C-MRSA strains are usually resistant to oxacillin, kanamycin, tetracycline, and fusidic acid (28), and it is particularly worrisome that we detected multidrug-resistant, PVL-positive MRSA causing hospital-acquired infections. Three of our PVL-positive MRSA isolates were resistant to both gentamicin and ofloxacin, drastically restricting treatment options. Two other PVL-positive MRSA isolates were resistant to ofloxacin but susceptible to gentamicin.

Among the C-MRSA isolates, a minor clone (ST37, *agr* type 3, SCC*mec* IVA) was detected in two patients and was found to harbor the *tst* gene. TSST-1-positive C-MRSA strains with an *agr*

type 2 ST5 background have been linked to the neonatal toxic shock-like exanthematous diseases in Japan, while TSST-1-positive C-MRSA strains with an ST5 SCC*mec* II background have been linked to toxic shock syndrome in Belgium (16).

Five of the 13 PVL- and TSST-1-negative H-MRSA isolates were *agr* type 1, SCC*mec* type III, and ST239, ST241, or ST637. These characteristics resemble those of the Brazilian and Hungarian MRSA clones (ST239 and *agr* type 1). Six H-MRSA isolates were *agr* type 2, SCC*mec* type IV or IVA, and ST5, thus resembling the MRSA pediatric clone (2–4). The last two H-MRSA isolates were *agr* type 3, SCC*mec* IV, and ST80 or ST635 (a single variant of ST80). Both were PVL negative but were related to the major PVL-positive clone of ST80. As PVL is encoded by phages, these two isolates may have lost the PVL genes (21).

These results show a very high prevalence of PVL-positive MRSA in Mustapha Pacha Hospital in Algiers. These strains were resistant to multiple antibiotics, including gentamicin and ofloxacin. The treatment options in the case of multiple-antibiotic-resistant MRSA strains included cotrimoxazole or pristinamycin for minor infections and glycopeptides for severe infections. Poor infection control procedures in this institution may have enabled community-acquired ST80 MRSA to supplant true hospital-acquired MRSA (ST5, ST239, ST241, and ST637). Local physicians must be informed of the emergence of such highly virulent strains and must also be aware of their implications for empirical treatment.

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